Current and Future Cell Therapy for Type 1 Diabetes

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General method of intrahepatic islet transplantation

Robertson RP. Diabetes 2010;59:1285-1291

Indications for islet transplant


• Clarke score (0 = no hypoglycemia, ≥4 = hypoglycemia unawareness), HYPO score is composite measure based on 4 weeks of records and a year historical review (median was 143, 25th to 75th interquartile range 46-423, and the 90th centile 1047. The lability index is based on changes in glucose leviers over time – 4 weeks.

Total number of recent islet allograft infusions per recipient per donor in CITR-participating centers


Comparison of rates of progress of alloislet vs. pancreas transplant for T1D

- First successful alloislet transplant reported 1980.
- First successful pancreas transplant reported 1966.

Rates of insulin independence after allogeneic ITA and IAK, annually after last infusion

Barton FB et al. Diabetes Care 2012;35:1436-1445
Islet transplant centers with 5-year insulin independence rates

<table>
<thead>
<tr>
<th>Center</th>
<th>Approach</th>
<th>2005</th>
<th>2006</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minneapolis</td>
<td>Anti-CD3 + immunosuppressant</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Minneapolis/CTSR</td>
<td>T cell depletion + antibody</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
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<tr>
<td>Edmonton</td>
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<td>100%</td>
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<tr>
<td>UCSF</td>
<td>ATG + anti-CD3 + immunosuppressant</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
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<td>100%</td>
<td>100%</td>
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<tr>
<td>UCI</td>
<td>Tac/MMF/SRL + immunosuppressant</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
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<td>100%</td>
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<tr>
<td>La Jolla</td>
<td>Tac/MMF/SRL + immunosuppressant</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
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<tr>
<td>Geneva, GACIE</td>
<td>ATG + Tac/MMF/SRL</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
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</tbody>
</table>

Legend: 5-year insulin independence rates updated from previous publications to include data from a blinded, international, multi-center study. Data presented at the American Transplant Congress Symposium “Advances in Islet Transplantation” June 6, 2012, Boston, USA, and at the International Islet and Pancreas Transplant Association (IPITA) meeting 2011, Prague, Czech Republic.


Islet transplant centers utilizing single-donor islet protocols

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<th>Year</th>
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<tbody>
<tr>
<td>Minneapolis</td>
<td>Anti-CD3 + immunosuppressant</td>
<td>2005</td>
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<td>Edwards</td>
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<td>Tac/MMF/SRL + immunosuppressant</td>
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<td>UC Irvine</td>
<td>Tac/MMF/SRL + immunosuppressant</td>
<td>2008</td>
</tr>
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Abbreviations: ATG – antithymocyte globulin, MMF – mycophenolate mofetil, SRL – sirolimus, UIC – University of Illinois at Chicago.

ITA Patient COH-016: Example of long-term benefits of islet transplant

- Insulin free (5 years, 26 days)

Islet cell transplantation: resolution of hypoglycemia

City of Hope results: Islet transplantation eliminates hypoglycemia.

Intrahepatic islets do not have normal responses to hypoglycemia

- Non-diabetic control subjects show plasma glucagon responses to insulin-induced hypoglycemia during a constant hypoglycemic clamp.
- This response is absent in T1D patients as well as patients who have undergone successful intrahepatic allograft or auto-islet transplant.
Recovery of initially absent glucagon responses to hypoglycemia after prolonged fasting and hepatic glycogen depletion

Liver glycogen depletion caused by prolonged fasting results in restoration of the glucagon response to hypoglycemia in intrahepatic islets in rats. Absence of glucagon response recurs after refeeding. This abnormality in alpha-cell function does not occur in fed animals if islets are placed into nonhepatic sites.

Comparison of intensive glucose management vs. islet transplantation in adult type 1 diabetics

Comparison of intensive glucose management vs. islet transplantation in adult type 1 diabetics

Islet injury and loss before and after transplantation in type 1 diabetics

Inset photograph shows human islets stained with dithizone red dye, indicative of a highly pure preparation. Lower inset labels indicate the challenges involved with early islet damage post-transplant, and the factors leading to late islet graft loss—both of which must be addressed to maintain excellent long-term graft function.

Biomarkers In Islet Transplantation

City of Hope islet transplantation diagnostic platform

Pre-transplant Islet Quality Assessment
- Laser Scanning Cytometry (LSC)
- Glucose-Responsive Oxygen Consumption Rate (OCR)
- Gene Signature of Islet Quality

Post-transplant In Vivo Monitoring of Islet Engraftment and Early Injury
- Bi-directional Pyrophosphorolysis – Activated Polymerization Assay (BiPAP)
- Methylation-Specific PCR (MSP)
Laser scanning cytometry

Cellular Composition

Cellular Health

• Significant increase in expression of inflammation and cell death markers in beta cells with aging

Caspase3
BBC3
Caspase4
TLR
IAPP

Example: Significant increase in expression of inflammation and cell death markers in beta cells with aging

Laser scanning cytometry

Effects of age on beta cell parameters

There is currently limited information in the field on aging in the non-diabetic human and rodent models.


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Glucose-responsive oxygen consumption rate (OCR)

Sweet et al. 2008

Glucose-responsive oxygen consumption rate (OCR)

Gene signature of islet quality

“Gene signature” for islet quality assessment

Gene expression analysis of 59 individual human islet preparations was performed and used to correlate the ability of each sample to reverse diabetes after transplantation in diabetic NOD SCID mice (Good islets vs. Bad islets). 199 genes were associated with islet quality.

A subset of 10 genes was independently assessed in 16 new islet preparations by Quantitative PCR. These 10 genes exhibited differential expression based on reversal of diabetes in mice (p<0.05)

Gene Good mean Bad mean Fold P value
NOTCH2 0.458 1.087 2.27 0.00006
MAP3K5 0.325 0.618 1.90 0.00034
RND3 0.479 0.898 1.87 0.00526
CARD6 0.038 0.062 1.64 0.00791
ITGB6 0.148 0.337 2.27 0.00936
IFITM2 1.730 2.767 1.60 0.01448
KCNMA1 2.016 1.357 1.48 0.02373
MPZL2 0.257 0.428 1.66 0.02898
MYOF 0.521 0.963 1.85 0.03750
SEPT9 2.722 3.760 1.38 0.04863

ROC Analysis of Combined 10 genes

Sensitivity%
86% Predictive Accuracy for reversal of diabetes in mice

Bidirectional pyrophosphorolysis-activated polymerization assay (BIPAP)

Monitoring of islet engraftment using genetic markers

A set of 22 assays that specifically distinguish the donor’s DNA from the recipient’s DNA

Establishing gene signatures of the highest quality islets for successful transplant outcomes

Donor genetic signature in blood of islet recipients reflects cell destruction.

Early detection of islet destruction would allow for protective interventions.
1. Donor specific markers were tracked in 103 sequential recipient plasma DNA samples from 11 transplant procedures.
2. Donor DNA marker detected in all patients immediately after transplantation for a mean duration of 22 days (15-28).
3. Sixteen donor DNA secondary signals were detected in four islet transplant patients, 13 of which (81%) were associated with events related to islet graft injury.

Biomarkers in islet transplantation

1. Islet Quality Assessment:
   - LSC (Laser Scanning Cytometry)
   - OCR (Oxygen Consumption)
   - IGS (Gene Signature)

2. Monitoring transplanted islets:
   - BiPAP (SNP in Genome)
   - MSP (Methylation pattern)

Model for Islet Therapy and Islet Scoring (MITRIS)

Early detection of islet injury with MSP

Methylation-Specific PCR
- Specific pattern of DNA methylation in insulin-producing cells
- DNA released into blood when cells die

Human MSP assay was developed based on the methylation pattern of the insulin promoter.

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Model for Islet Therapy and Islet Scoring (MITRIS)

Relationship of calculated insulin deficit (CID) with % HgbA1c and daily reported blood glucose

Orr et al. unpublished
Other problems with islet transplantation

- **Shortage of donor islets from cadaveric pancreas**
  - Large quantities of islets, typically from multiple (2-3) donors, have been needed to achieve insulin independence; larger initial infusion leads to larger mass of surviving beta cells
  - If lower islet masses from single donors could achieve insulin independence, islet transplantation could reach a wider audience and also reduce multiple exposure to HLA antigens
  - There is a growing need for a renewable source of beta cells (from stem cells or xenotissue)

- **Requirement for lifelong immunosuppression**
  - Further increases risks for cardiovascular disease, atherosclerosis, dyslipidemia, and cancer
  - Tacrolimus can cause neuro- and nephrotoxicity and is additionally toxic to beta cells; elimination of tacrolimus may improve metabolic function but increases risk of rejection
  - There is a growing need for immunomodulation to reduce dependence on immunosuppression

Researching solutions for the future advancement of islet transplantation

- **In vivo islet expansion**
- **Stem cells**
- **Xeno-transplantation**
- **Alternative sites of transplantation**
- **Immune modulation**

**Gastrin** is a strong promoter of islet neogenesis both in vitro and in vivo that may work synergistically with GLP-I. Therefore, administration of Gastrin to islet recipients may induce graft neogenesis and insulin independence with fewer islets.

Growth factors (such as TGF-β, epidermal growth factor, and keratinocyte growth factor) and gastrointestinal peptides (such as glucagon-like peptide-1 and gastrin) could stimulate β-cell neogenesis.

Stem cells

- **Advantages:**
  - Circumvents shortage of islet donors with a renewable source of cells
  - Autologous cells harvested, expanded, and injected back into the same patient avoid issues with graft rejection and eliminates the need for immunosuppression

- **Disadvantages:**
  - Undifferentiated cells pose risk of teratoma formation
  - Ethical issues with human embryonic stem cells
Generation of Renewable Beta Cell Source
Characteristics of Our Functional Human ESC Maturation Protocol

Enriching for human insulin expressing cells.
Glucose-responsive insulin-secreting cells are generated in culture.

Production of safe, affordable and accessible responsive insulin-producing cells for patient treatment.

Xenotransplantation

- Advantages:
  - Ability to transplant well-differentiated cells that are responsive to glucose
  - Potential for genetic modification focuses treatment on the donor rather than the recipient

- Disadvantages:
  - If islet survives Immediate Blood Mediated Inflammatory Reaction (IBMIR), will then have to circumvent T-cell mediated cellular (xenograft) rejection
  - Graft-versus-host disease

Alternative sites to portal vein infusion

- Anticoagulation with heparin has reduced the impact of IBMIR, improving rate of single-donor engraftment from 10% to 40% (Koh et al. 2010), but bleeding, bile leakage, and portal vein thrombosis are still risks of percutaneous portal vein infusion

- Alternative sites to the liver would circumvent monitoring issues; ready access to the site would enable graft monitoring, implantation, and biopsy.

- In choosing a new site, islets ideally require high oxygen tension and rich vascular supply, as well as means to deliver insulin to tissues

- Biological scaffolds and encapsulating methods may help decrease exposures to IBMIR-related destruction and improve the metabolic microenvironment to support islets

Promising alternative sites
islet/stem cell transplantation

Immune modulation strategies: promoting regulation
Stem cell education therapy
In vitro expansion of T-reg
Mixed chimerism
Mesenchymal stem cells

IAK Patient COH-022: Case Summary
IAK Patient COH-022: Case Summary

Intrahepatic Transplant of a single donor islet graft failed within 3 months due to islet duct obstruction (Koh et al 2010)

Good response following two islet infusions given approx 1 mo apart, followed by rapid islet graft failure. Transient islet DSA & other evidence of alloresponse detected. Renal graft stable.
Natural history of the immunopathogenesis of T1DM

Stem Cell Educator therapy using a closed-loop system

Markers of immune function in T1D patients after Stem Cell Educator therapy

Improvement of β-cell function by Stem Cell Educator therapy

Questions

Regulatory T Cell Control of Human Type 1 Diabetes Clinical Trials to Treat Diabetic Patients
Clinical trials of Treg in T1D

- Cellular therapy of T1D with ex vivo expanded CD4+CD25+CD127− Treg (ISRCTN06128462)
  - Investigator: Dr. Piotr Trzonkowski (Poland)
  - Subjects: Male and female, recently diagnosed T1D, 5-18 yo
  - Dosage: 30 x 10^6 cells/kg b.w. (22.5 x 10^8 / 75 kg)
  - Phase I safety trial of CD4+CD127lo/-CD25+ polyclonal Treg adoptive immunotherapy for the treatment of T1D (NCT01210664)
  - Investigators: Drs. Gitelman, Bluestone, Herold (U.S.)
  - Subjects: Male and female, recently diagnosed T1D, 18-45 yo
  - Dosage: 0.05-26 x 10^8
  - Estimated completion date: December 2016


Mixed chimerism can be induced by classical bone marrow transplantation (HCT) or by a novel method developed by the Zeng lab at COH.

Approaches for induction of mixed chimerism

- Classical Bone Marrow Transplantation
  - The anti-CD3 antibodies specifically target recipient T cells that are responsible for graft rejection.
  - The radiation-free anti-CD3 conditioning is relatively non-toxic.

- Induction of Chimerism via Classical HCT
  - Causes Graft vs Host Disease (GVHD)

- Radiation-Free Anti-CD3 Conditioning:
  - Anti-CD3 Preconditioning Targeting Recipient T-Cells

Results:
- Donor islets transplanted into the liver or under the renal capsule failed after chimerism induction reversed diabetes for >100 days.

Future of islet and stem cell transplantation research

- Currently, >80 trials of islet transplant are registered with Clinicaltrials.gov
- >1500 potentially enrollable subjects
- At an estimated cost of >$150,000 USD per subject, the burden on research organizations is enormous (> $227 million USD) and limits trial progression
- Approval of islet transplantation as standard of care has been obtained in Canada, Europe and Australia but not in USA. Obtaining a biological license would make it reimbursable
- Stem cell transplant has recently begun in the USA, awaiting establishing its safety

Mesenchymal stem cells

- Attractive for transplantation for many reasons
  - Derived from a variety of adult human tissues, including adipose tissue, umbilical cord or umbilical cord blood, fibroblasts, endometrium, liver cells, but bone marrow is the richest source.
  - High capacity to self-replicate and differentiate both in vitro and in vivo to bone and fat-forming cells and other tissues.
  - Maintain capacity of multi-lineage differentiation potential even after prolonged culture conditions.
  - Possess immune suppressive qualities and are considered "immunologically inert".
  - When co-transplanted with other adult stem cells, MSCs facilitate engraftment of hematopoietic stem cells.

Stable mixed chimerism was induced in WF rats by intra-portal infusion of bone marrow cells, MSCs, and non-islet pancreatic cells from fully allogeneic donors.

Results:
- Donor islets transplanted into the liver or under the renal capsule failed after chimerism induction reversed diabetes for >100 days.

Islet allograft tolerance induced by co-infusion of non-islet pancreatic tissue, mesenchymal stem cells and bone marrow cells

Y. Mullen et al (COH) 2012, unpublished data

Mesenchymal stem cells (MSCs) are pluripotent stromal cells that can give rise to cells of diverse lineages.

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